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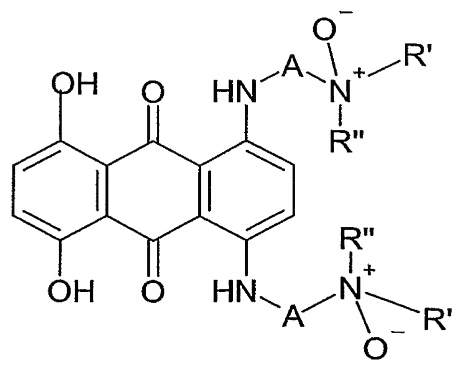
(54) **Title:** FORMULATIONS OF ANTHRAQUINONE DERIVATIVES



(I)

(57) **Abstract:** A stable, sterile aqueous solution of a compound of formula (I): in which A is a C alkylene group with a chain length between NH and N(O)R'R" of at least 2 carbon atoms and R' and R" are each separately selected from C_{1-4} alkyl groups and C_{2-4} hydroxyalkyl and C_{2-4} dihydroxyalkyl groups, or R' and R" together are a C_{2-6} alkylene group, is formulated in a unit dosage form in a sealed container, said solution having a concentration of the compound of formula (I) up to 150 mg/ml and a pH in the range of 5 to 9. The solution may be prepared without a freeze drying step.

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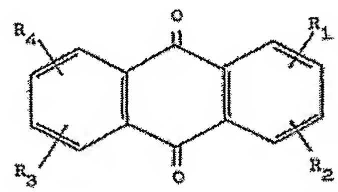
FORMULATIONS OF ANTHRAQUINONE DERIVATIVES

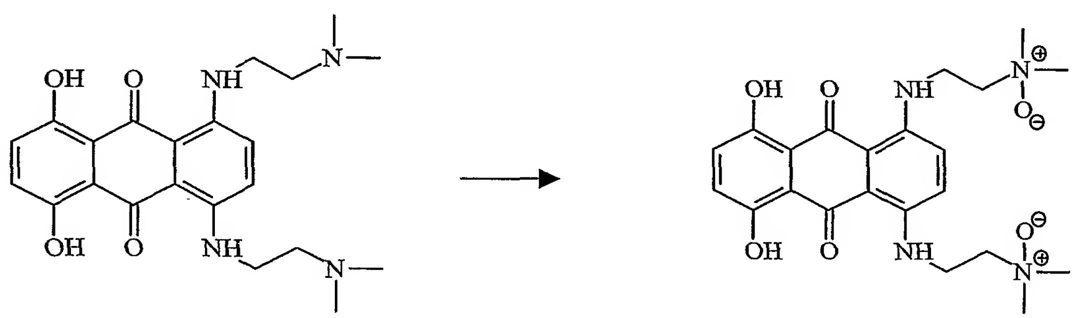
The invention relates to novel formulations of anthraquinone derivatives such as AQ4N, a bis-bioreductive agent with value in the treatment of cancer.

WO-A-91/05824 (National Research Development Corporation) discloses a compound of formula:



in which R_1, R_2, R_3 and R_4 are each separately selected from hydrogen, X, NH–A–NHR and NH–A–N(O)R'R" wherein X is hydroxy, halogeno, amino, C_{1-4} alkoxy or C_{2-8} alkanoyloxy, A is a C alkylene group with a chain length between NH and NHR or N(O)R'R" of at least 2 carbon atoms and R, R' and R" are each separately selected from C_{1-4} alkyl groups and C_{2-4} hydroxyalkyl and C_{2-4} dihydroxyalkyl groups in which the carbon atom attached to the nitrogen atom does not carry a hydroxy group and no carbon atom is substituted by two hydroxy groups, or R' and R" together are a C_{2-6} alkylene group which with the nitrogen atom to which R' and R" are attached forms a heterocyclic group having 3 to 7 atoms in the ring, the compound optionally being in the form of a physiologically acceptable salt.

A preferred compound within this general formula is the bis-*N*-oxide AQ4N, (banoxantrone) which may be synthesised by oxidation of AQ4:



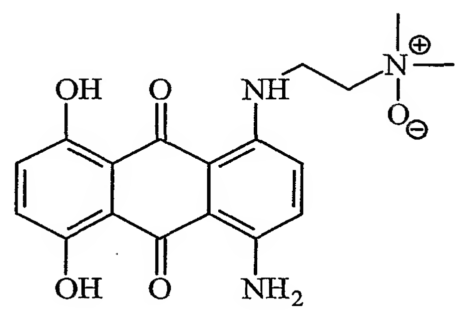
AQ4 AQ4N

AQ4N is in fact a prodrug and the reverse reaction occurs *in vivo*, reductive metabolism in hypoxic cancer cells giving the active agent, AQ4, in its protonated form. The prodrug is relatively non-toxic when compared with the active agent, AQ4, making it particularly attractive for administration as a pharmaceutical. However, it

does not readily give a crystalline form. Up to now, the thinking has been that it is desirable to prepare and formulate AQ4N for administration in the form of a salt or freeze dried material.

AQ4N has been reported in the form of a dihydrochloride salt AQ4N.2HCl. See for example *J. Chem. Soc., Perkin Trans. I*, 1999, 2755–2758 (Lee *et al.*) and WO-A-00/05194 (BTG International Limited). However, investigations of AQ4N.2HCl raw material have demonstrated significant quantities of an impurity 1-amino-4-{[2-(dimethylamino)ethyl]amino}-5,8-dihydroxyanthraquinone, denoted AQMN, which has been characterised by LCMS and also confirmed by synthesis of a genuine sample of AQMN material:

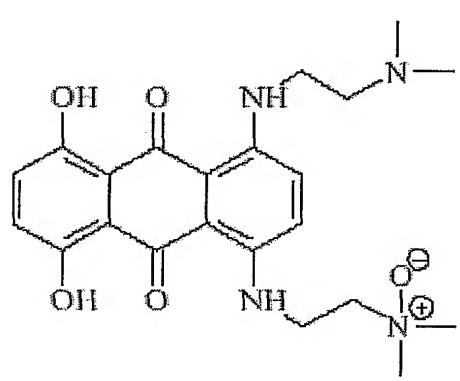
AQMN



This impurity can be formed by degradation of AQ4N, and more significantly shows an undesirable level of cytotoxicity, generally being higher than that of AQ4N itself. This level of cytotoxicity is to be avoided in a compound which is intended to be administered in the form of a relatively non-toxic prodrug.

Although the AQMN degradation is the predominant pathway, a further degradation product of AQ4N under acidic and neutral aqueous solution conditions is a further undesirable product, namely the mono-*N*-oxide, AQ4M:

AQ4M

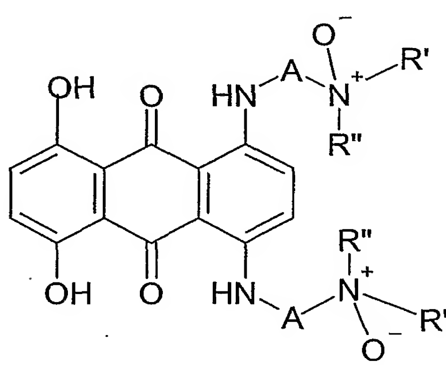


Co-pending patent application WO-A-03/078387 (BTG International Limited) discloses further salts of AQ4N, with a physiologically acceptable acid having a pKa in the range of – 3.0 to 9.0. The patent application also discloses the formulation of the compound so that upon dissolution in aqueous solution the pH of the solution is in the range of 5 to 9.

After manufacture of a pharmaceutical product, the point at which it expires (and can no longer be used in patients) is defined by the decrease in the content of the intended drug and/or the increase in associated impurities. A reduction in the degradation rate of the parent compound increases the time it takes for that compound to reach the point at which it expires (the expiry date). Where required, drug compounds are stabilised by producing the final product as a freeze dried formulation.

However with AQ4N the present inventors have found that the stability and manufactured quality of AQ4N as a freeze dried formulation is inversely related to the water content of the freeze dried cake: that is, the less water, the poorer the quality and stability of the final product. Moreover, it is more stable as a solution (at the appropriate pH) than as the equivalent freeze dried formulation. Thus it is better to produce AQ4N without freeze drying.

Thus according to the present invention there is provided a stable, sterile aqueous solution of a compound of formula (I):



(I)

in which A is a C alkylene group with a chain length between NH and N(O)R'R" of at least 2 carbon atoms and R' and R" are each separately selected from C_{1-4} alkyl groups and C_{2-4} hydroxyalkyl and C_{2-4} dihydroxyalkyl groups in which the carbon atom attached to the nitrogen atom does not carry a hydroxy group and no carbon atom is substituted by two hydroxy groups, or R' and R" together are a C_{2-6} alkylene

group which with the nitrogen atom to which R' and R" are attached forms a heterocyclic group having 3 to 7 atoms in the ring,

in a unit dosage form in a sealed container, said solution having a concentration of the compound of formula (I) up to 150 mg/ml and a pH in the range of 5 to 9.

Preferably the pH of the solution is in the range of 5.0 and 8.4, more preferably 6.0 to 8.0, with a pH between 7.0 and 8.0 being optimal. When the compound of formula (I) is AQ4N, a pH 7.4 has been shown to be the most stable. This contrasts with the freeze dried AQ4N formulation, where the most stable was observed to be pH 6.

Preferably the compound of formula (I) is present at a concentration of between 0.1 and 100 mg/ml.

The compound of formula (I) may be previously isolated in the form of a physiologically acceptable salt which will be an acid addition salt with an organic or inorganic acid. Preferably the physiologically acceptable acid has a pK_a in the range of – 3.0 (minus 3.0) to 9.0, and more preferably in the range of 2.0 to 9.0. More preferably the physiologically acceptable acid has a pK_a in the range of 2.0 to 6.0.

Preferably the physiologically acceptable acid is selected from the group consisting of tartaric acid, malonic acid, dichloroacetic acid, citric acid, maleic acid, benzenesulfonic acid, pimelic acid and acetic acid.

More preferably the physiologically acceptable acid has a pK_a in the range of 3.0 to 6.0. The physiologically acceptable acid may especially be an organic acid, particularly an organic mono- , di- or tri-acid, and especially one selected from the group consisting of tartaric acid, citric acid, pimelic acid and acetic acid.

The group A in formula (I) may be branched but is conveniently a straight chain alkylene group, i.e. tetramethylene, especially trimethylene, or particularly ethylene.

R' and R" may also have a branched carbon chain but are conveniently straight chain whether they are alkyl groups or hydroxy-substituted alkyl groups. When R' or R" is a monohydroxyalkyl group this is conveniently substituted terminally and when R' or R" is a dihydroxyalkyl group this is conveniently substituted terminally by one of the hydroxy groups. When R' and R" are alkyl the preference is for a group of three or especially two or one carbon atoms and when R' and R" are hydroxy-substituted alkyl the preference is for the alkyl group to be of three carbon atoms or, in the case

of a monohydroxyalkyl group, alternatively of two carbon atoms. Examples of preferred individual groups R' and R" are CH_3, CH_2CH_3, $CH_2CH_2CH_3$, CH_2CH_2OH, $CH_2CH_2CH_2OH$, $CH(CH_3)CH_2OH$ and $CH_2CHOHCH_2OH$.

R' and R" will more usually be identical.

Alternatively, as indicated, R' and R" together with the nitrogen atom to which they are attached may represent a heterocyclic group —$N(CH_2)_n$ where n is 2 to 6, i.e. aziridin-1-yl, azetidin-1-yl, pyrrolidin-1-yl, piperidin-1-yl and perhydroazepin-1-yl, the smaller groups such as azetidin-1-yl and especially aziridin-1-yl being of most interest.

Specific groups NH–A–N(O)R'R" of particular interest are $NH–(CH_2)_2–N(O)(CH_3)C_2H_5$, $NH–(CH_2)_2–N(O)(C_2H_5)_2$, $NH–(CH_2)_2–N(O)(CH_2CH_2OH)_2$, $NH–(CH_2)_2–N(O)(CH_2CH_2CH_2OH)_2$, $NH–(CH_2)_2–N(O)(CH(CH_3)CH_2OH)_2$, $NH–(CH_2)_2–N(O)(CH_2CHOHCH_2OH)_2$ and especially $NH–(CH_2)_2–N(O)(CH_3)_2$.

The physiologically acceptable salt when simply dissolved in aqueous solution will normally give a solution having a pH lower than the desired range. For example, the acetate salt of AQ4N in a 1.4 millimolar solution aqueous solution has a pH of 3.8. Thus preferably the compound of formula (I) is formulated in a mixture containing additional components so the pH of the solution is buffered to be in the range of 5 to 9.

A buffer is a solvated mixture of salt and acid, which oppose changes in pH when small amounts of acid and bases are added to the solution. Suitable buffers include sodium acetate buffer and sodium orthophosphate buffer.

The salt with a physiologically acceptable acid may be prepared by any conventional means, for example by reaction of the organic base (I) with the appropriate inorganic or organic acid, usually by simple admixture in solution. The acid addition salts are generally crystalline solids which are relatively soluble in water, methanol, ethanol and similar solvents. One salt form may also be converted into another by chromatography using a column which has been pre-treated with the desired physiologically acceptable acid.

The compound of formula (I) may be formulated with a physiologically acceptable diluent or carrier for use as pharmaceuticals for both veterinary and particularly human use by a variety of methods. For instance, it may be applied as a composition incorporating a liquid diluent or carrier, for example an aqueous

solution, suspension or emulsion, which may often be employed in injectable form for parenteral administration and therefore may conveniently be sterile and pyrogen free. Oral administration may also be used.

Other types of administration than by injection or through the oral route which are of use in both human and veterinary contexts include the use of suppositories or pessaries. Another form of pharmaceutical composition is one for buccal or nasal administration or alternatively drops for administration into the eye which may conveniently contain a sterile liquid diluent or carrier. Other formulations for topical administration include lotions, ointments, creams, gels and sprays.

Compositions may be formulated in unit dosage form, i.e. in the form of discrete portions containing a unit dose, or a multiple or sub-unit of a unit dose.

Whilst the dosage of the compound used will vary according to the activity of the particular compound and the condition being treated, it may be stated by way of guidance that a dosage selected in the range from 25–500 mg/m^2 per day, particularly in the range from 50–300 mg/m^2 per day, will often be suitable although higher doses than this, for example in the range from 25–750 mg/m^2 per day, or even doses up to 1200 mg/m^2, may be considered in view of the lower level of toxic side effects obtained with the compounds (I). This dosage regime may be continued for however many days is appropriate to the patient in question, the daily dosages being divided into several separate administrations if desired. Thus, for example, in the case of conditions such as advanced breast cancer, non-Hodgkin's lymphoma and hepatoma, treatment for one day followed by a repeated dose after an interval, such as 21 days, may be appropriate whilst for the treatment of acute non-lymphocytic leukaemia, treatment over 5 consecutive days may be more suitable. Alternatively, single administrations spaced by several days, for example one dose every two or three weeks, may be used.

The compounds (I) are of particular value for the treatment of cancer in warm blooded animals including humans. The compounds are of interest in relation to the treatment of solid tumours, such as various forms of sarcoma and carcinoma, and also for disseminated tumours such as leukaemias. Areas of particular interest are the treatment of breast cancer, lung cancer, prostate cancer, pancreatic cancer, and oesophageal cancer, and the treatment of non-Hodgkin's lymphoma and acute non--lymphocytic leukaemia. In the treatment of cancer, parenteral and sometimes topical administration is often of particular interest. Moreover, it may be advantageous to use

the compounds (I) in a combined treatment, given separately or together in the same composition, with other anti-cancer agents, such as mitotic inhibitors, for example vinblastine; alkylating agents, for example cisplatin, carboplatin and cyclophosphamide; other antimetabolites, for example 5-fluorouracil, cytosine arabinoside and hydroxyurea; intercalating antibiotics, for example adriamycin and bleomycin; enzymes, for example asparaginase; topoisomerase inhibitors, for example etoposide and biological response modifiers, for example interferon. The compounds (I) may also be used in combined treatment with radiation therapy of the tumour.

The present invention thus includes a method for aiding regression and palliation of cancer which comprises administering to a patient a therapeutically effective amount of a compound (I) as defined hereinbefore.

In addition to their anti-cancer use the compounds (I) are of interest for various other pharmaceutical applications in view of their activity as chelating agents.

The present invention also includes a process for the preparation of a solution in a unit dosage form in a sealed container as defined above, comprising introducing a stable, sterile aqueous solution of the compound of formula (I) into a container and sealing the container, in which the solution is prepared without a freeze drying step.

The invention is illustrated by the following Examples in which—

Figure 1 shows the first derivative of pH *versus* pH in a solution of AQ4N dihydrochloride;

Figure 2 shows the first derivative of pH *versus* NaOH molar equivalence under the same conditions;

Figure 3 shows the increase in AQMN over incubation time in 5 mg/ml solutions incubated at 40 °C for 14 days;

Figure 4 shows the increase in AQMN over incubation time in 5 mg/ml solutions incubated at 40 °C for 63 days;

Figure 5 shows the decrease in AQ4N over incubation time in 5 mg/ml solutions incubated at 40 °C for 14 days;

Figure 6 shows the decrease in AQ4N over incubation time in 5 mg/ml solutions incubated at 40 °C for 63 days;

Figure 7 shows the decrease in AQ4N over incubation time at different pH values and concentrations;

Figure 8 shows the increase in AQMN over incubation time at different pH values and concentrations;

Figure 9 shows the AQ4N content of freeze dried formulations against time when stored at 4 °C;

Figure 10 shows the AQ4N content of freeze dried formulations against time when stored at 25 °C;

Figure 11 shows the AQ4N content of freeze dried formulations against time when stored at 40 °C;

Figure 12 shows changes in the AQ4M content in different freeze dried batches at 4 °C;

Figure 13 shows changes in the AQMN content in different freeze dried batches at 4 °C;

Figure 14 shows changes in the AQ4M content in different freeze dried batches at 25 °C;

Figure 15 shows changes in the AQMN content in different freeze dried batches at 25 °C;

Figure 16 decrease in AQ4N content of freeze dried and solution formulations against time when stored at 55 °C;

Figure 17 shows the increase in AQMN content of solution formulations against time when stored at 4 and 55 °C;

Figure 18 shows the increase in AQ4M content of solution formulations against time when stored at 4 and 55 °C;

Figure 19 shows the increase in AQMN content of solution formulations against time when stored at 4 °C;

Figure 20 shows the increase in AQMN content of solution formulations against time when stored at 25 °C;

Figure 21 shows how AQMN increase is observed in two ways;

Figure 22 shows the increase in AQMN content of freeze dried formulations against time in several batches when stored at 4 °C;

Figure 23 shows the degradation rate (as measured by the slope of AQMN increase when fitted to a linear plot) against the water content of the batches at 4 °C; and

Figure 24 shows the step increase in AQMN level during manufacture against the water content of the batches at 4 °C.

EXAMPLES

Example 1: Demonstration of the effect of pH on the physico-chemical properties of AQ4N

Changes in the pH of a solution of AQ4N dihydrochloride were monitored to demonstrate the degradation of AQ4N into AQMN. The pH curves are shown in Figures 1 and 2. Figure 1 shows a clear dissociation at between pH 7.7 and pH 9.4, and this equates to the dissociation events shown in Figure 2 at approximately 2 molar equivalence. A low pH dissociation event can be observed, speculatively assigned to a pH between 4.1 and 4.6 where the molar equivalence is between 0.95 and 1.15.

Example 2: Demonstration of the cytotoxicity of AQMN

The toxicity of a pure sample (99.3%) in the P388 system of AQ4N and AQMN were determined and the results obtained are presented in Table 2.

Table 2: AQ4N and AQMN cytotoxicity values

Compound	IC_{50} P388 (nM)	Relative toxicity (normalised to AQ4N)
AQ4N	410	1.0
AQMN	77	5.2

Based on these data, AQMN has a cytotoxicity which is at least 5 times greater than that of AQ4N in the same system. The "greater than" modifier is required since all samples of AQ4N contain substantial percentages of AQMN, which will affect the toxicity result.

Example 3: Demonstration of the instability of AQ4N dihydrochloride in solution—accumulation of AQMN

The degradation of AQ4N was investigated using 5 mg/ml solutions of AQ4N at a pH of 2.4, 4.5 and 6.8, which equated to water, 20 mM sodium acetate buffer and 20 mM sodium orthophosphate buffer, respectively. The primary degradation pathway of AQ4N is its conversion to AQMN. The increase in AQMN concentration in 5 mg/ml solutions incubated at 40 °C over an intermediate time period (14 days) is shown in Figure 3.

The degradation rates equate to a 0.84% (w.r.t. AQ4N), 0.19% (w.r.t. AQ4N) and 0.02% (w.r.t. AQ4N) increase in AQMN content per day under these conditions.

Using linear regression and cross correlating with the known quantity of AQMN in the material used as a standard these data indicate the accumulation rates of AQMN presented in Table 3.

Table 3: Accumulation of AQMN

	H_2O	Acetate	PO_4
AQMN increase per day	0.84%	0.19%	0.02%

A similar trend was observed in the data after 63 days, as shown in Figure 4. The rate of accumulation (calculated in the same way as above) shows that in the phosphate buffer AQMN increases by 0.6% per month.

Example 4: Demonstration of the instability of AQ4N dihydrochloride in solution—degradation of AQ4N

The effect of pH on the stability of AQ4N was determined by investigating AQ4N degradation in different solutions. The solutions chosen were again distilled water, 20 mM sodium acetate buffer (pH = 4) and 20 mM sodium phosphate buffer (pH = 7). After preparation of the 5 mg/ml AQ4N buffered solutions the pH was corrected to the required pH of the buffer. The final pH values were 2.4, 4,5 and 6.8 for the distilled water, 20 mM sodium acetate buffer and 20 mM sodium phosphate buffer, respectively. The samples were incubated at 40 °C and sampled at regular intervals. Assay was carried out by sample dilution followed by HPLC analysis.

After 14 days an interim analysis was carried out with the data being shown in Figure 5.

The solutions were further incubated at 40 °C for a total of 63 days. The final graph is shown in Figure 6. The values obtained for the AQ4N contents are consistent with the quantities of AQ4N weighed into the original samples (within experimental error).

Example 5: Demonstration of the instability of AQ4N as a 40 mg/ml solution in 10 mM sodium phosphate buffer at pH = 7.4 to 'standard' autoclave conditions

A solution of AQ4N was prepared at 40 mg/ml in 10 mM sodium phosphate buffer at pH = 7.4. Samples of this solution were analysed before and after a standard

autoclave cycle (121 °C for 15 minutes). The post autoclave samples showed a decrease in AQ4N concentration of 7.3%. Corresponding increases were seen with associated impurities.

Example 6: Demonstration of the relationship between the stability of AQ4N and pH at various concentrations

Several solutions of AQ4N were prepared as in sodium phosphate buffer (10 mM) with different concentrations of AQ4N at different pH values and the results obtained are presented in Table 4.

Table 4: pH of AQ4N solutions

Solution	AQ4N conc. (mg/ml)	Solution pH at preparation
pH 5 1 mg	1.0	5.14
pH 6 1 mg	1.0	6.18
pH 7 1 mg	1.0	7.03
pH 8 1 mg	1.0	8.43
pH 7 10 mg	10.0	7.48
pH 7 40 mg	39.8	7.48

The samples were assayed before incubation and then placed in a 55 °C incubator. The solutions were re-sampled after one day, one week, two weeks, four weeks and eight weeks.

The results in terms of the AQ4N content are shown in Figure 7 and the results in terms of the increase in the AQMN in Figure 8.

These results show that between 10 and 40 mg/ml (at pH 7.4) there is little effect of concentration change on the degradation rate. These results also show that the stability pH profile of AQ4N runs as follows:

$$7.4 \text{ (stable)} < (7.0 \cong 6.2) < (8.4 \cong 5.1) \text{ (unstable)}$$

Example 7: Demonstration of the in stability of AQ4N as a freeze dried formulation at various pH values

Three lyophilised batches of AQ4N (40 mg/ml, sodium phosphate buffer 10 mM at pH = 6.0, 7.0 and 8.0) were manufactured as BN99019, BN99020 and BN99021, respectively. Samples for these batches were stored at 4 °C, 25 °C and 40 °C and analysed at various time points according to Table 5.

Table 5: Time points of stability batches (BN99019, BN99020 and BN99021) under different storage temperatures

Batch		99019			99020			99021		
Temp.		4 °C	25 °C	40 °C	4 °C	25 °C	40 °C	4 °C	25 °C	40 °C
Time point (months)	**Time point (days)**									
0	0	✓	✓	✓	✓	✓	✓	✓	✓	✓
1	34	✓	✓	✓	✓	✓	✓	✓	✓	✓
3	111	✓	✓	✓	✓	✓	✓	✓	✓	✓
6	188	✓	✓	✓	✓	✓	✓	✓	✓	✓
9	271	✓	✓	✓	✓	✓	✓	✓	✓	✓
12	383	✓	✓	✓	✓	✓	✓	✓	✓	✓
18	559	✓	✓	N/A	✓	✓	N/A	✓	✓	N/A
24	756	✓	✓	N/A	✓	✓	N/A	✓	✓	N/A
36	1106	✓	✓	N/A	✓	✓	N/A	✓	✓	N/A

At each time point AQ4N content, AQ4N related impurity levels, water content, pH and osmolality were investigated. The latter three are not further discussed.

The AQ4N content levels for the pH = 6.0, 7.0 and 8.0 formulations as BN99019, BN99020 and BN99021, respectively, are shown against time in Figures 9 to 11 for individual temperatures. Figure 9 shows the AQ4N content when stored at 4 °C, Figure 10 at 25 °C. and Figure 11 at 40 °C.

The degradation of the AQ4N (as measured by the decrease of the parent compound) can clearly be seen at 25 and 40 °C. BN99019 (pH = 6.0) appears to be the most stable.

The related impurity levels were plotted for three batches under the 4 and 25 °C storage conditions.

The degradation profile of the AQ4N manufactured product indicate that the main degradants are AQ4M and AQMN. The related impurity levels for the pH = 6.0, 7.0 and 8.0 formulations as BN99019, BN99020 and BN99021, respectively, are shown in Figures 12 to 15. Figures 12 and 13 respectively show the AQ4M and AQMN changes at 4 °C, and Figures 14 and 15 show the AQ4M and AQMN changes at 25 °C.

The 25 °C results confirm that the pH = 6.0 formulation is the most stable. However, it appears as if the degradant levels in the BN99019 samples stored at 4 °C are slightly higher than the pH = 7.0 and 8.0 formulations.

Example 8: Demonstration of the relationship between the stability of AQ4N and pH at 40 mg/ml

Previous work on the stability of AQ4N at low concentrations (5 mg/ml) (see Examples 3 and 4 above) suggested that at neutral pH the stability of the AQ4N molecule is high. This experiment was designed to investigate how stable the formulated AQ4N product is as a reconstituted formulation (at 40 mg/ml). Formulated AQ4N material was prepared at 40 mg/ml in 10 mM sodium phosphate buffer at pH 6.0, 7.0 and 8.0 and freeze dried. These batches were placed on stability for 3 years as an indication of the stability of the freeze dried material (see Example 7). After that time the vials stored at 4 °C (where the loss of AQ4N was show to be minimal) were reconstituted in 2 ml of distilled water. (The pH of the last time point on stability was 5.916, 6.946 and 7.928, respectively.) Two vials were assayed for AQ4N, AQMN and other related impurities after reconstitution. Both freeze dried and reconstituted vials were stored as specified in Tables 6 to 8.

Table 6: Formulated solutions at pH 6.0

Time (weeks)	4 °C (solution)	55 °C (solution)	55 °C (f/d vial)
4	-	2 vials	2 vials
9	1 vials	2 vials	2 vials
13	2 vials	2 vials	2 vials

Table 7: Formulated solutions at pH 7.0

Time (weeks)	4 °C (solution)	55 °C (solution)	55 °C (f/d vial)
4	-	2 vials	2 vials
9	2 vials	2 vials	2 vials
13	2 vials	2 vials	2 vials

Table 8: Formulated solutions at pH 8.0

Time (weeks)	4 °C (solution)	55 °C (solution)	55 °C (f/d vial)
4	2 vials	2 vials	2 vials
9	2 vials	2 vials	2 vials
13	2 vials	2 vials	2 vials

The results for AQ4N content for the samples stored at 55 °C are shown in Figure 16.

These data confirm that at pH 6.0 the freeze dried material is more stable and the equivalent pH 7 or 8 material, but also shows that formulated AQ4N is more stable as a solution than as a freeze dried preparation. The pH stability profile for solutions of AQ4N in this experiment is shown to be:

$$(6.9 \cong 7.9)\ (\text{stable}) < 5.9\ (\text{unstable})$$

The results for the degradants (AQMN and AQ4M) are shown in Figures 17 and 18 for the solutions samples.

A direct numerical comparison of the degradation rates of the Examples 9 and 6 is not possible since different AQ4N raw materials were used as standards and samples for these individual experiments.

Example 10: Demonstration of effect of freeze drying (against solution preparation) on the quality of AQ4N product

Formulated AQ4N material was prepared at 40 mg/ml in 10 mM sodium phosphate buffer at pH 7.0. A portion of this solution was filled into ampoules and the remained into vials and freeze dried according to the cycle as specified in Tables 9 and 10.

Table 9: Temperature Profile

Stage	Temperature (°C)	Time			Cumulative Time HH.MM.SS*
		Hours	Minutes	Seconds	
00	20	3	00	00	00.00.00
01	– 40	2	00	00	03.00.00
02	– 40	5	00	00	05.00.00
03	– 5	15	00	00	10.00.00
04	– 10	5	00	00	25.00.00
05	10	5	00	00	30.00.00
06	10	0	00	00	35.00.00
	Total Time =	35	00	00	

* Cumulative time at start of programme section

Table 10: Cycle positions

Cycle Position	Time		
	Hours	Minutes	Seconds
Initiation	00	00	01
Freezing	4	00	00
Evacuation	0	29	58
Primary Drying	23	30	00
Secondary Drying	7	00	00
Cycle End	0	00	01
Total Time =	35	00	00

The AQMN content of the samples before and after freeze drying (ampoule fill and vial fill, respectively) were measured as shown in Table 11.

Table 11: AQMN content

Sample	AQMN content (% peak area w.r.t. std.)
Ampoule	22.2%
Vial	35.2%

The AQMN content of the relevant AQ4N raw material compared to the standard is 22.6%. This indicates that the AQMN is generated during the freeze drying cycle, and the elimination of the lyophilisation step improves the overall quality of the product.

Example 11: Demonstration of the effect of temperature on the stability of AQ4N

The AQ4N solution (liquid-filled) ampoules prepared in Example 10 were tested for stability by incubation at 4 °C and 25 °C. At various time points the AQ4N, AQMN and related impurity contents of the vials were assayed by HPLC. The AQMN contents for the ampoules (designated BN03-05) stored at 4 °C are shown in Figure 19 as compared to the AQ4N freeze dried vials (BN03-06), also prepared in Example 10 (and stored at 4 °C). The AQMN contents for the ampoules stored at 25 °C are shown in Figure 20 as compared to the equivalent AQ4N freeze dried vials

(BN99020, pH = 7), prepared in Example 7, and stored at 25 °C. (The data for both groups are only shown for comparable times at approximately 200 days.)

It should be noted that since BN03-05 and BN99020 were prepared from different raw material batches (and so contained different levels of AQMN initially) the *y*-axis is expressed as a percentage of the initial AQMN content of the final product batch).

Example 12: Demonstration of the stability of AQ4N at at 4 °C

A further illustration of the stability of the aqueous formulation was demonstrated by an additional batch of AQ4N solution (liquid-filled) ampoules (BN03-12) prepared in a similar way to those in Example 10. However, BN03-12 was prepared at with a larger volume 200 mg in 5 ml of solution. The batch was placed at 4 °C and analysed at various timepoints to allow suitable expiry date extension. The AQMN levels of this material were consistent with Example 11, where the AQMN content value (w.r.t. the standard) increased from 0.226 after manufacture to 0.232 after 162 days at 4 °C.

Example 13: Demonstration of the instability of AQ4N as a freeze dried formulation with respect to water content

Several batches of the AQ4N freeze dried product (40 mg/ml, 10 mM sodium phosphate buffer at pH = 7.0) were prepared in a similar or identical manner to that described above.

AQMN increase is observed in two ways: a step increase in AQMN content during manufacture (see Example 10 above) and gradual increase over time (see Figure 21).

The AQMN increase at 4 °C in several batches is shown in Figure 22.

The straight lines on the graph represent the AQMN level found in the raw material which was used to manufacture particular batches. (Raw material 1 was used for batches 01-13, 02-18 and 03-06. Raw material 2 was used for batches 99020, 00-15 and 01-10.)

The water content of the batches (as measured by Karl Fisher titration at QC / post manufacture testing) was plotted against the degradation rate at 4 °C (as measured by the slope of AQMN increase when fitted to a linear plot) and is given in Figure 23.

The water content of the batches (as measured by Karl Fisher titration at QC / post manufacture testing) was plotted against the step increase in AQMN level during manufacture (specifically freeze drying) and is given in Figure 24.

Both these graphs show the correlation between the degradation of the product (during and after manufacture) and the amount of water in the freeze dried cake after manufacture. This appears to be a function of freeze drying process itself which is indicated by the water content.

Example 14: Preparation of stable, sterile aqueous solutions

Preparation and qualification of equipment and environment

Equipment and containers for the production of the AQ4N final product were treated appropriately (including dry heat sterilisation, autoclave sterilisation and IPA disinfection) for aseptic manufacture of a sterile product. The clean rooms and laminar air flow cabinets used during the preparation and production were monitored (using settle and contact plates, air pressure differential and particle counts) in a manner appropriate for the manufacture of a sterile product. Balances used for weighing during the production process were calibrated with traceable weights immediately prior to manufacture.

Buffer preparation

Using a three-place balance, 3.450 g of sodium dihydrogen orthophosphate 1-hydrate (monobasic) was weighed into a 600 mL beaker and 8.900 g of di-sodium hydrogen orthophosphate 2-hydrate (dibasic) was weighed into a 1000 mL beaker. 400 mL of water for irrigation (WFI) was added to the monobasic and 800 mL to the dibasic beakers respectively. A magnetic follower was added and allowed to stir for 15 minutes. Each solution was aseptically transferred to an individual 500 mL (monobasic) and 1000 mL (dibasic) volumetric flask. Each flask was appropriately labelled, made to volume and stoppered.

390 mL of Monobasic solution and 610 mL of Dibasic solution were added to a 2000 mL beaker using 500 mL and 1000 mL measuring cylinders. These were mixed and labelled as 0.05 M phosphate buffer pH 7.0.

375 mL of the pH 7.0 buffer was transferred into a 500 mL volumetric flask by weight. (weight required is 376.58g since the density of the solution has been determined to be 1.0042 g/ml) and used for solubilisation of AQ4N.

Processing of the bulk solution

30.00g of AQ4N (as a dihydrochloride salt) was added into a 1000 mL beaker. The weight was recorded and a magnetic stirrer bar added.

43.5 mL of 2M sodium hydroxide was added via a 50 mL syringe and a 5 mL syringe into a 500 mL beaker and make to an approximately 150 mL volume with WFI. The 150 mL of the NaOH was added to the AQ4N powder. Approximately 225 mL of pH 7.0 buffer was added to the AQ4N mixture ensuring that all the AQ4N in the 1000 mL beaker was solubilised with buffer during this procedure. This solution was then transferred to the 2000 mL beaker, rinsed with the remaining buffer solution and allowed to stir for 15 minutes. The AQ4N solution was made up to approximately 600 ml volume with WFI. The pH was established and adjusted to 7.0 ± 0.1 if required. WFI was added to the AQ4N solution until a target weight of 760 grams was obtained.

Filtration of bulk solution

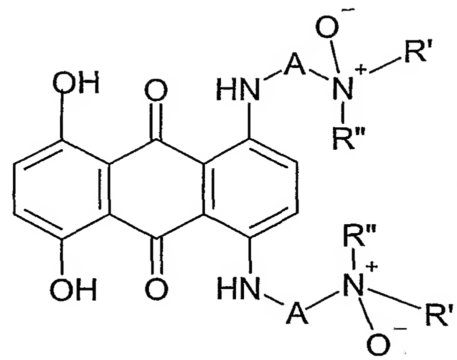
A peristaltic pump with clean, sterile, inert tubing was aseptically assembled to pump the AQ4N solution from the bulk solution, through two Millipak™ 20 filters and into a clean, treated, sterile Schott bottle assembly (including filling lines and vent filters).

Ampoule filling and stoppering

If ampoule filling was required, ampoules were purchased, prepared, treated and opened to a suitable quality standard for pharmaceutical use prior to AQ4N solution preparation. The filtered AQ4N solution was dispensed into ampoules using a peristaltic pump, with clean, sterile, inert tubing. Dispensing was carried out by volume, but checked by weight before filling and at regular intervals during manufacture (nominally after every fiftieth ampoule, but this would vary according to batch size.). The density of the solution was previously established. The ampoules were sealed manually by gas–air flame in the clean room.

CLAIMS

1. A stable, sterile aqueous solution of a compound of formula (I):



(I)

in which A is a C alkylene group with a chain length between NH and N(O)R'R" of at least 2 carbon atoms and R' and R" are each separately selected from C_{1-4} alkyl groups and C_{2-4} hydroxyalkyl and C_{2-4} dihydroxyalkyl groups in which the carbon atom attached to the nitrogen atom does not carry a hydroxy group and no carbon atom is substituted by two hydroxy groups, or R' and R" together are a C_{2-6} alkylene group which with the nitrogen atom to which R' and R" are attached forms a heterocyclic group having 3 to 7 atoms in the ring,

in a unit dosage form in a sealed container, said solution having a concentration of the compound of formula (I) up to 150 mg/ml and a pH in the range of 5 to 9.

2. A solution as claimed in claim 1 in which the pH of the solution is in the range of 5.0 and 8.4.

3. A solution as claimed in claim 2 in which the pH of the solution is in the range of 6.0 to 8.0.

4. A solution as claimed in any preceding claim in which the compound of formula (I) is present at a concentration of between 0.1 and 100 mg/ml.

5. A solution as claimed in any preceding claim in which A is a straight chain alkylene group.

6. A solution as claimed in claim in which A is ethylene.

7. A solution as claimed in any preceding claim in which R' and R" are straight chain alkyl groups or hydroxy-substituted alkyl groups.

8. A solution as claimed in claim 7 in which R' and R" are each CH_3 or CH_2CH_3.

9. A solution as claimed in claim 8 in which each group of formula NH–A–N(O)R'R" is group of formula NH–$(CH_2)_2$–N(O)$(CH_3)_2$.

10. A solution as claimed in any preceding claim formulated in a mixture containing additional components so that the pH of the solution is buffered to be in the range of 5 to 9.

11. A solution as defined in any preceding claim for use in therapy.

12. A process for the preparation of a solution as defined in any preceding claim comprising introducing a stable, sterile aqueous solution of the compound of formula (I) into a container and sealing the container, in which the solution is prepared without a freeze drying step.

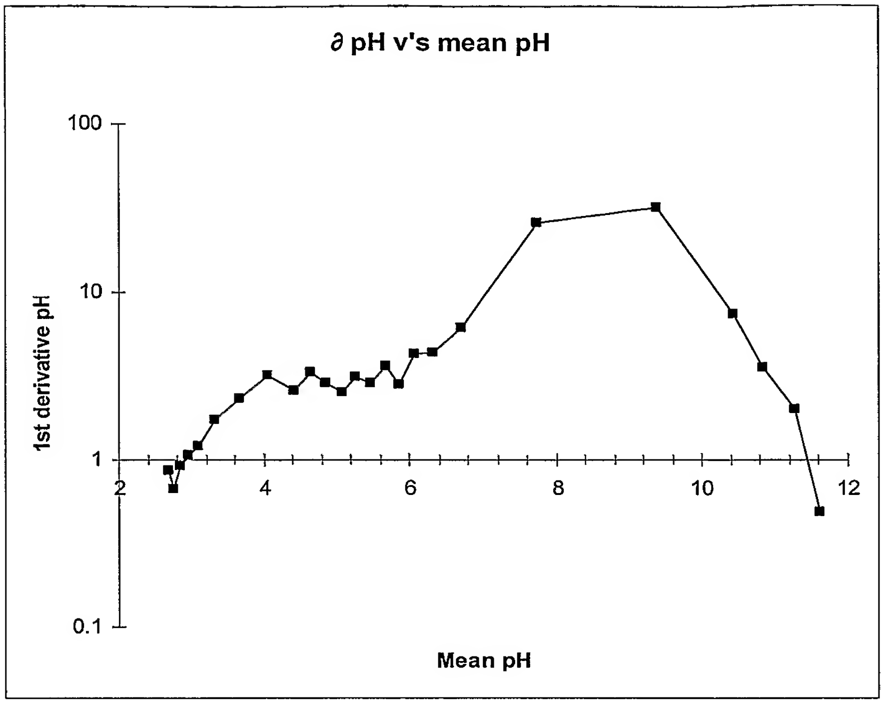

∂ pH v's mean pH
100
10
1
0.1
1st derivative pH
2
4
6
8
10
12
Mean pH

Fig. 1

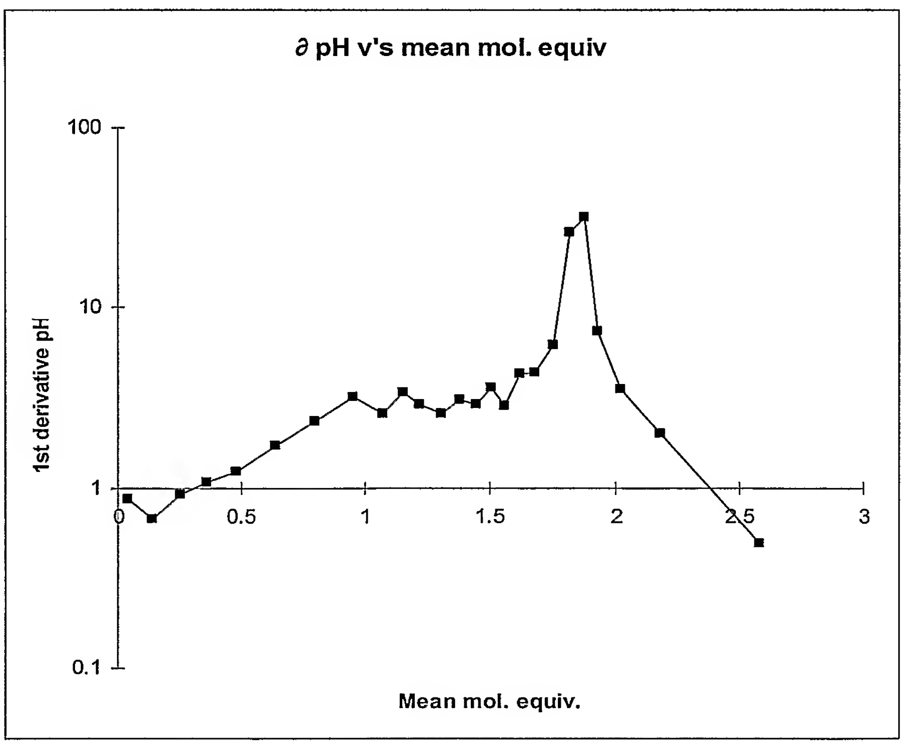

∂ pH v's mean mol. equiv
100
10
1
0.1
1st derivative pH
0
0.5
1
1.5
2
2.5
3
Mean mol. equiv.

Fig. 2

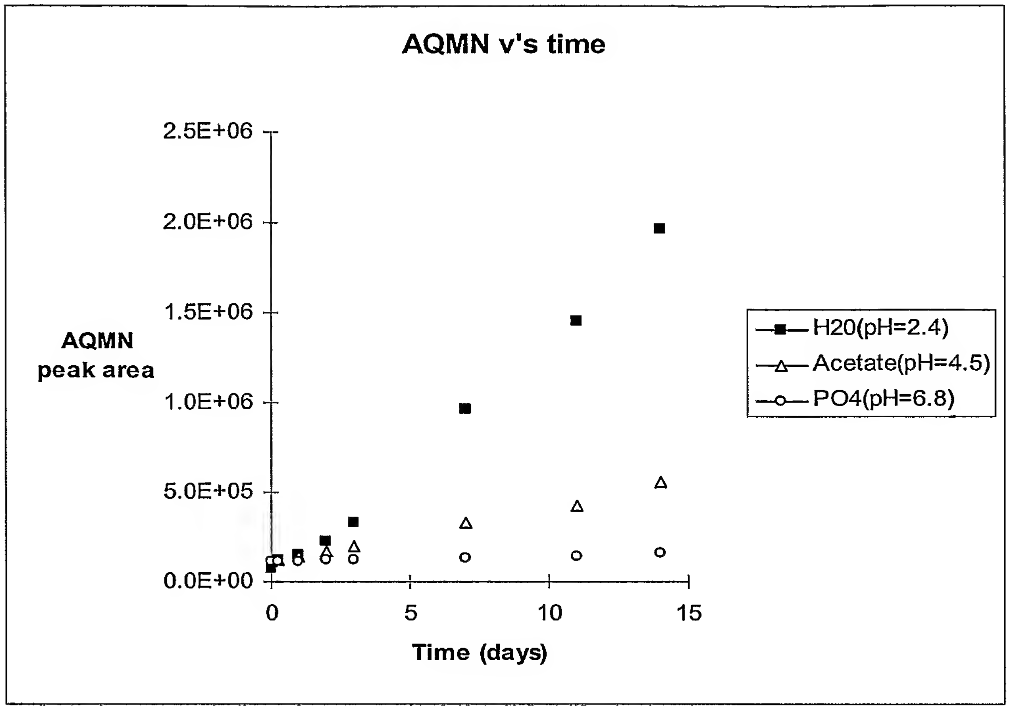

AQMN v's time
2.5E+06
2.0E+06
1.5E+06
1.0E+06
5.0E+05
0.0E+00
AQMN peak area
0
5
10
15
Time (days)
H20(pH=2.4)
Acetate(pH=4.5)
PO4(pH=6.8)

Fig. 3

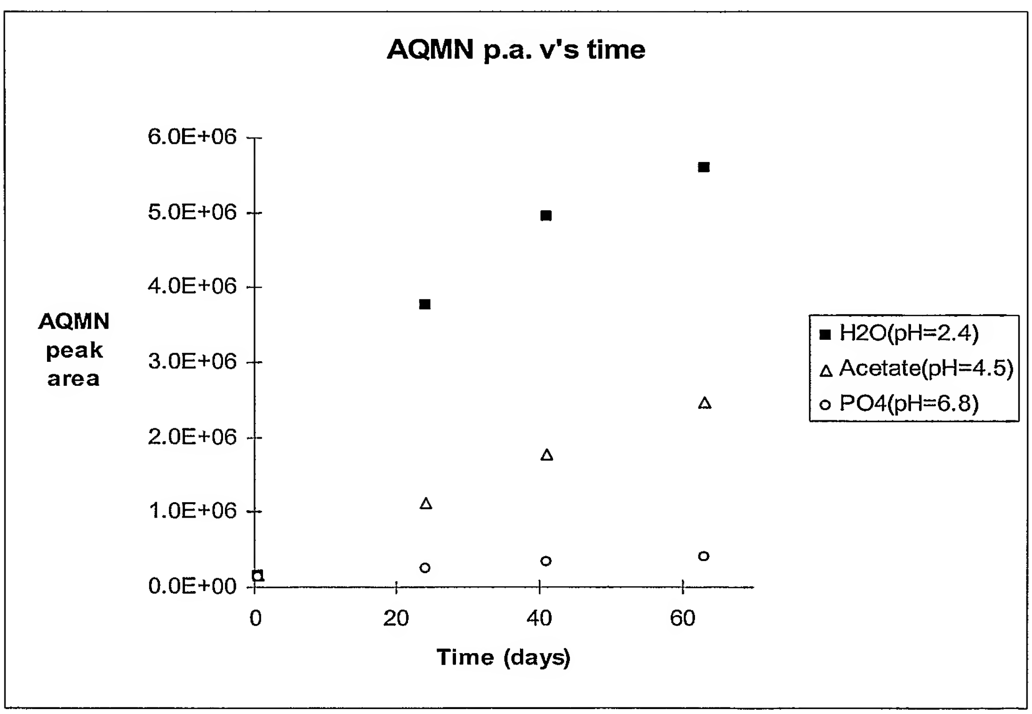

AQMN p.a. v's time
6.0E+06
5.0E+06
4.0E+06
3.0E+06
2.0E+06
1.0E+06
0.0E+00
AQMN peak area
0
20
40
60
Time (days)
H2O(pH=2.4)
Acetate(pH=4.5)
PO4(pH=6.8)

Fig. 4

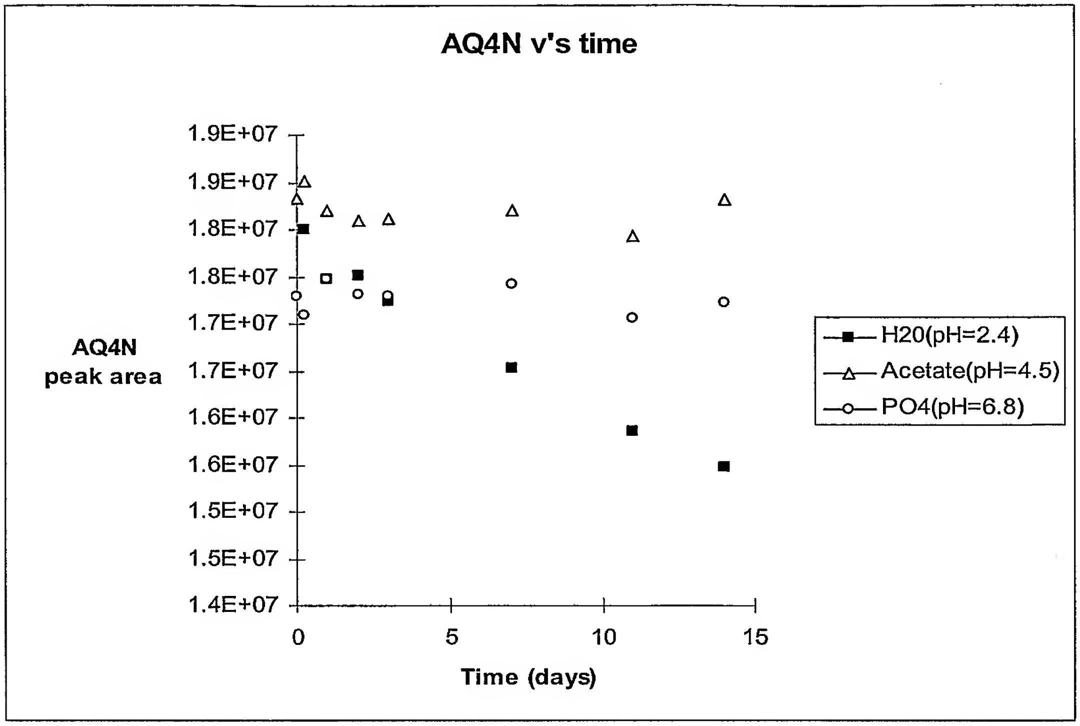

AQ4N v's time
AQ4N peak area
1.9E+07
1.9E+07
1.8E+07
1.8E+07
1.7E+07
1.7E+07
1.6E+07
1.6E+07
1.5E+07
1.5E+07
1.4E+07
0
5
10
15
Time (days)
H20(pH=2.4)
Acetate(pH=4.5)
PO4(pH=6.8)

Fig. 5

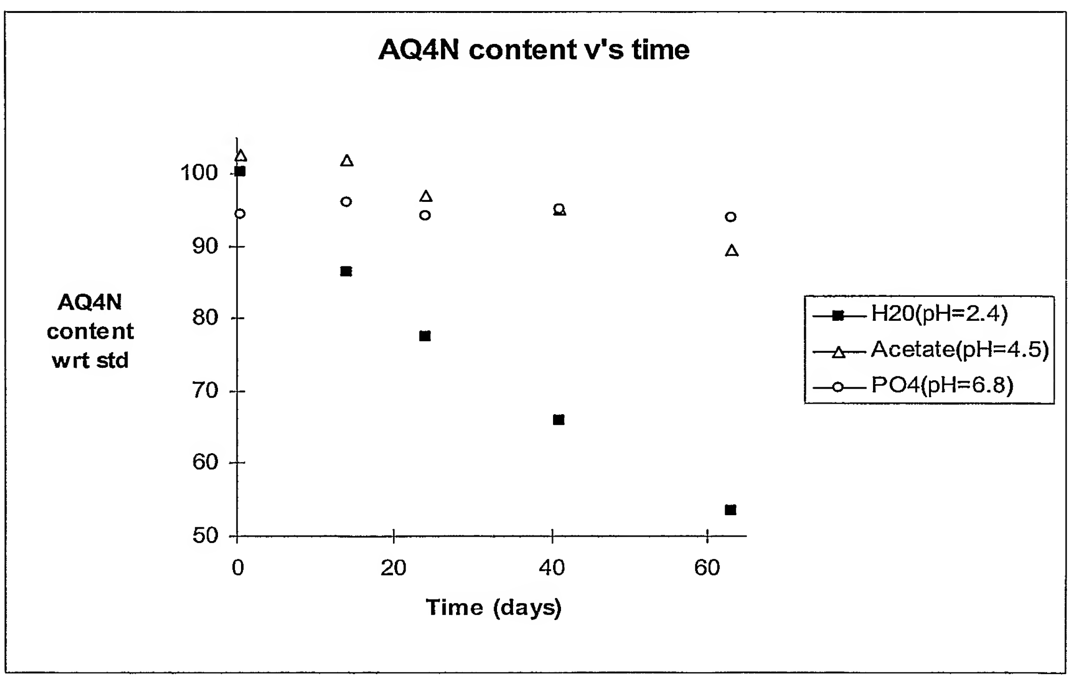

AQ4N content v's time
AQ4N content wrt std
100
90
80
70
60
50
0
20
40
60
Time (days)
H20(pH=2.4)
Acetate(pH=4.5)
PO4(pH=6.8)

Fig. 6

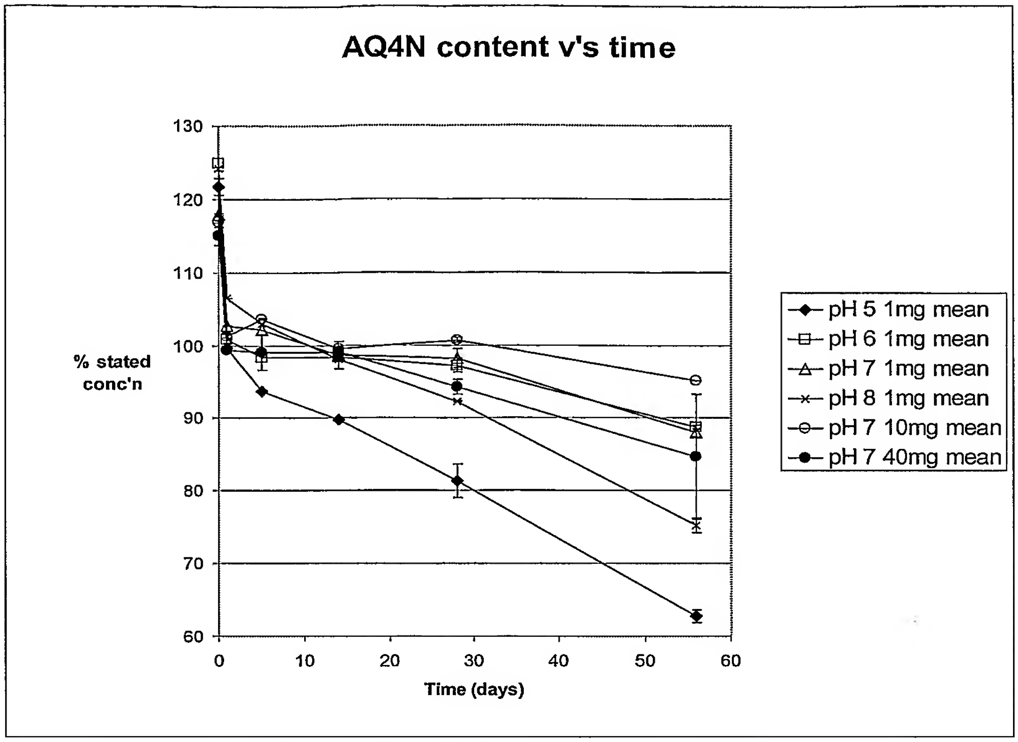

AQ4N content v's time
% stated conc'n
130
120
110
100
90
80
70
60
0
10
20
30
40
50
60
Time (days)
pH 5 1mg mean
pH 6 1mg mean
pH 7 1mg mean
pH 8 1mg mean
pH 7 10mg mean
pH 7 40mg mean

Fig. 7

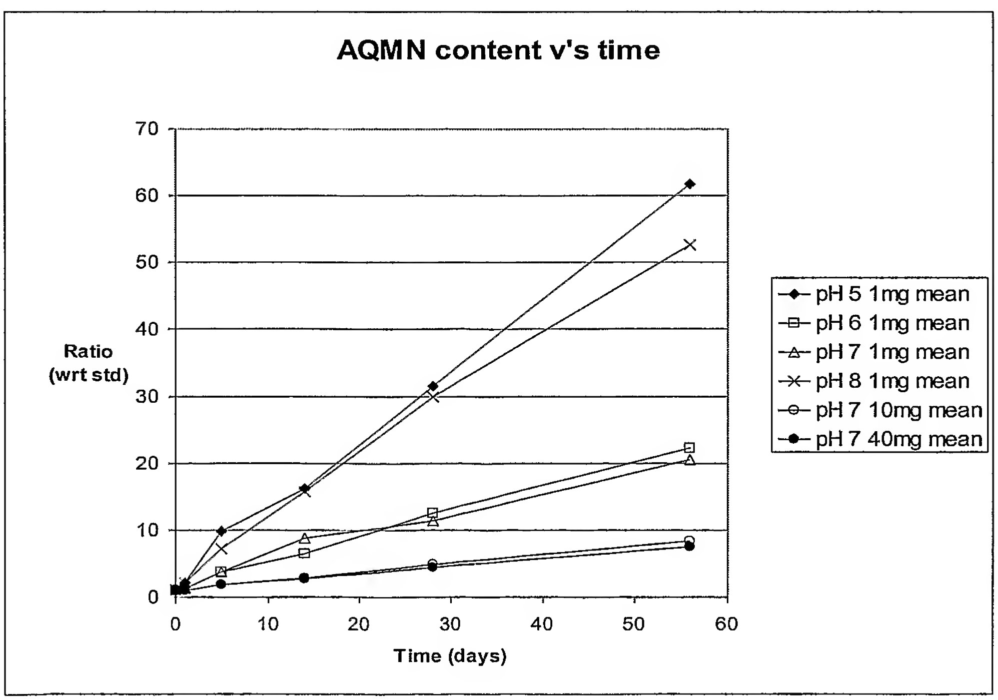

AQMN content v's time
Ratio (wrt std)
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
Time (days)
pH 5 1mg mean
pH 6 1mg mean
pH 7 1mg mean
pH 8 1mg mean
pH 7 10mg mean
pH 7 40mg mean

Fig. 8

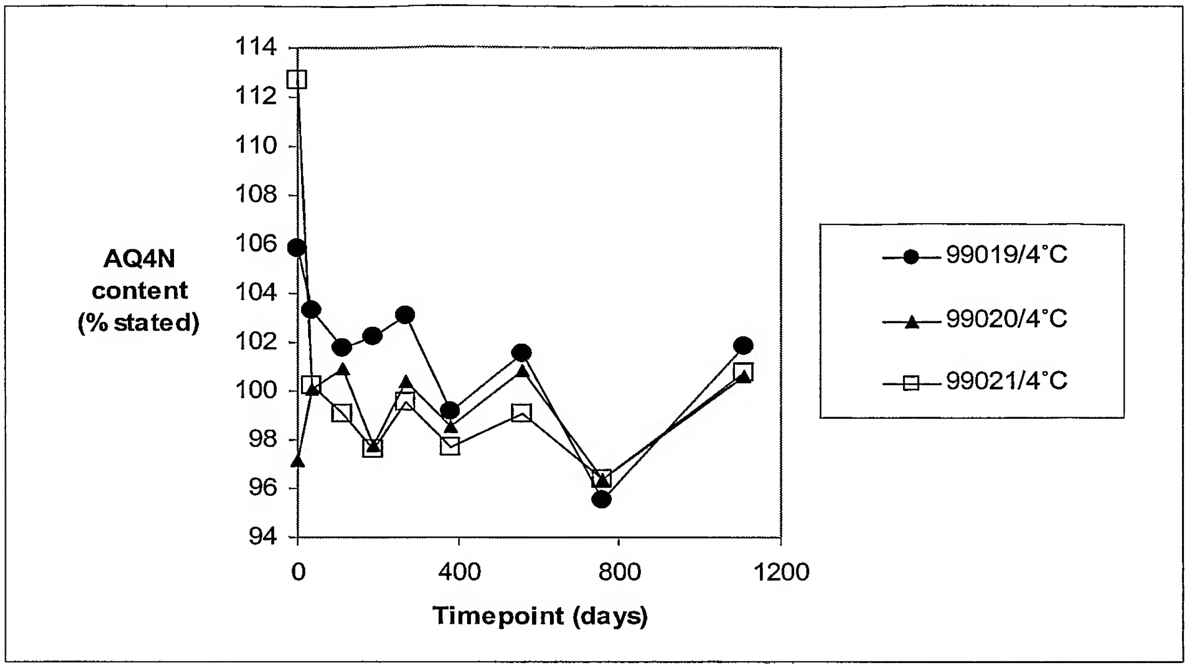

114
112
110
108
106
104
102
100
98
96
94
0
400
800
1200
AQ4N content (% stated)
Timepoint (days)
99019/4°C
99020/4°C
99021/4°C

Fig. 9

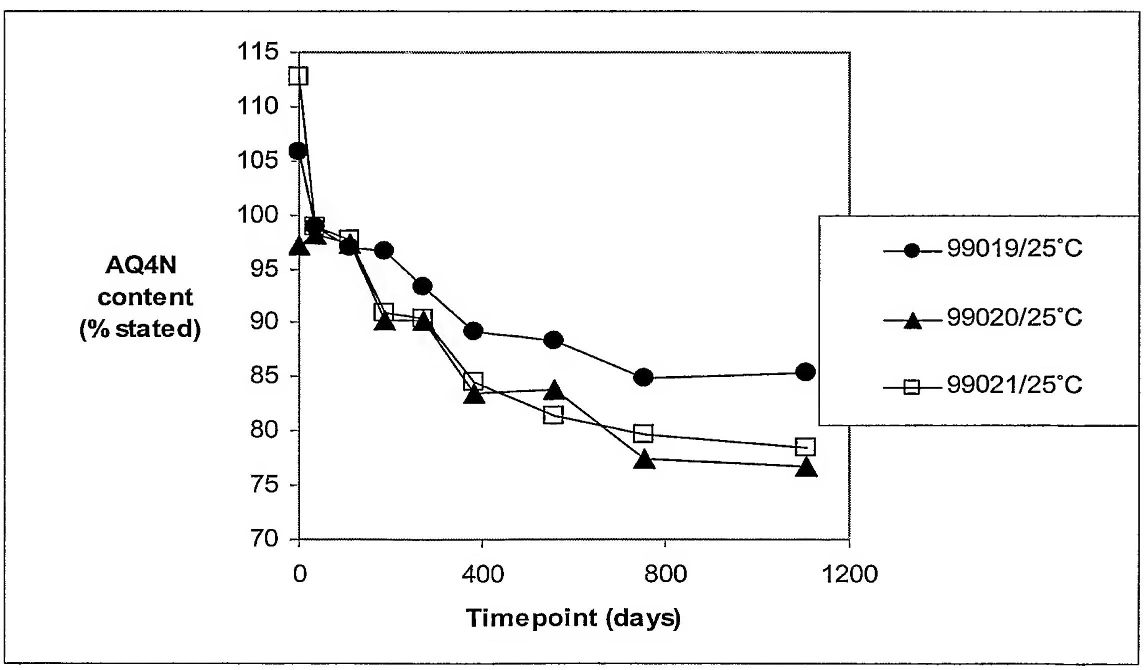

115
110
105
100
95
90
85
80
75
70
0
400
800
1200
AQ4N content (% stated)
Timepoint (days)
99019/25°C
99020/25°C
99021/25°C

Fig. 10

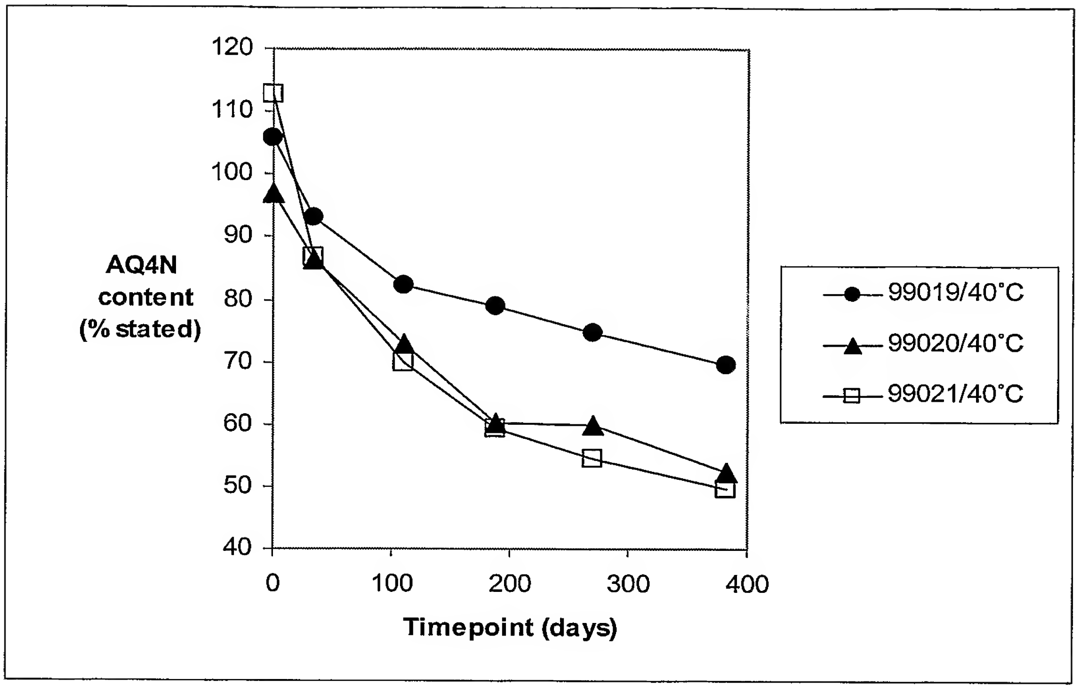

120
110
100
90
80
70
60
50
40
0
100
200
300
400
AQ4N content (% stated)
Timepoint (days)
99019/40°C
99020/40°C
99021/40°C

Fig. 11

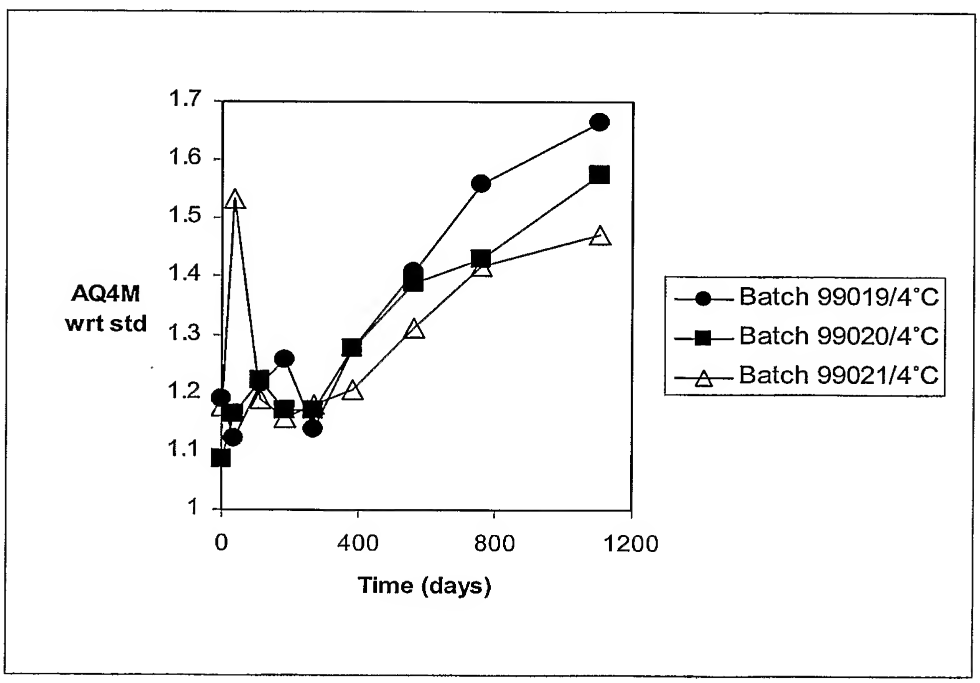

1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0
400
800
1200
AQ4M wrt std
Time (days)
Batch 99019/4°C
Batch 99020/4°C
Batch 99021/4°C

Fig. 12

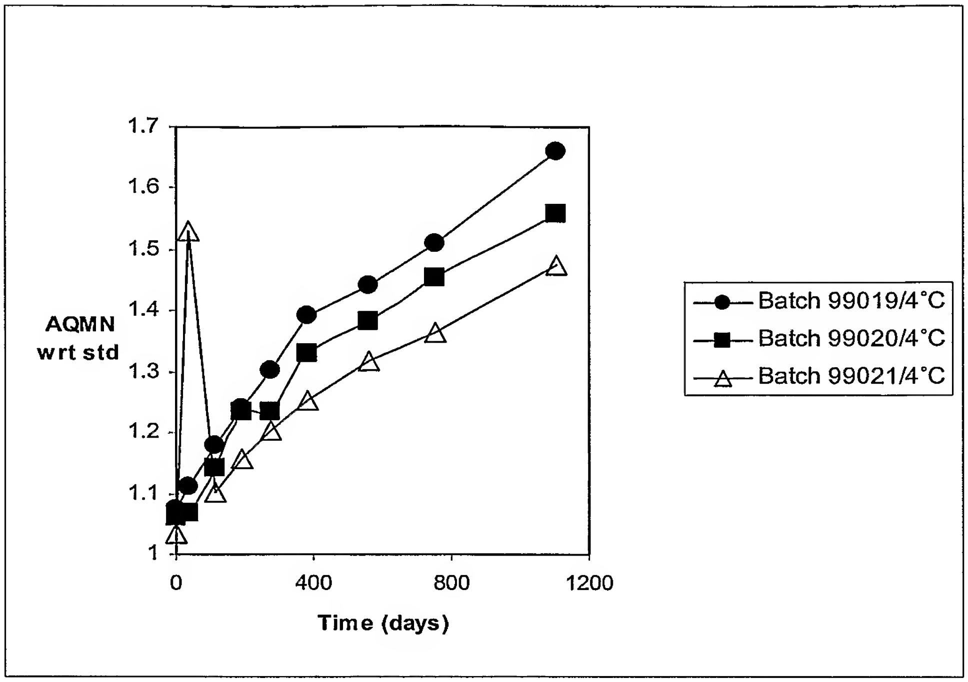

1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
AQMN
wrt std
0
400
800
1200
Time (days)
Batch 99019/4°C
Batch 99020/4°C
Batch 99021/4°C

Fig. 13

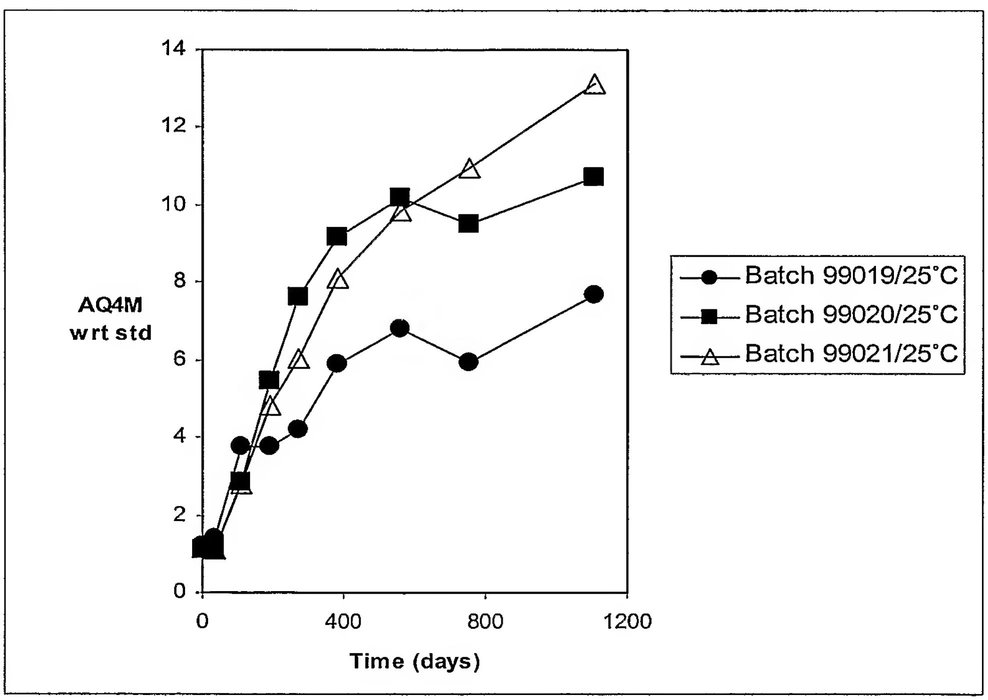

14
12
10
8
6
4
2
0
AQ4M
wrt std
0
400
800
1200
Time (days)
Batch 99019/25°C
Batch 99020/25°C
Batch 99021/25°C

Fig. 14

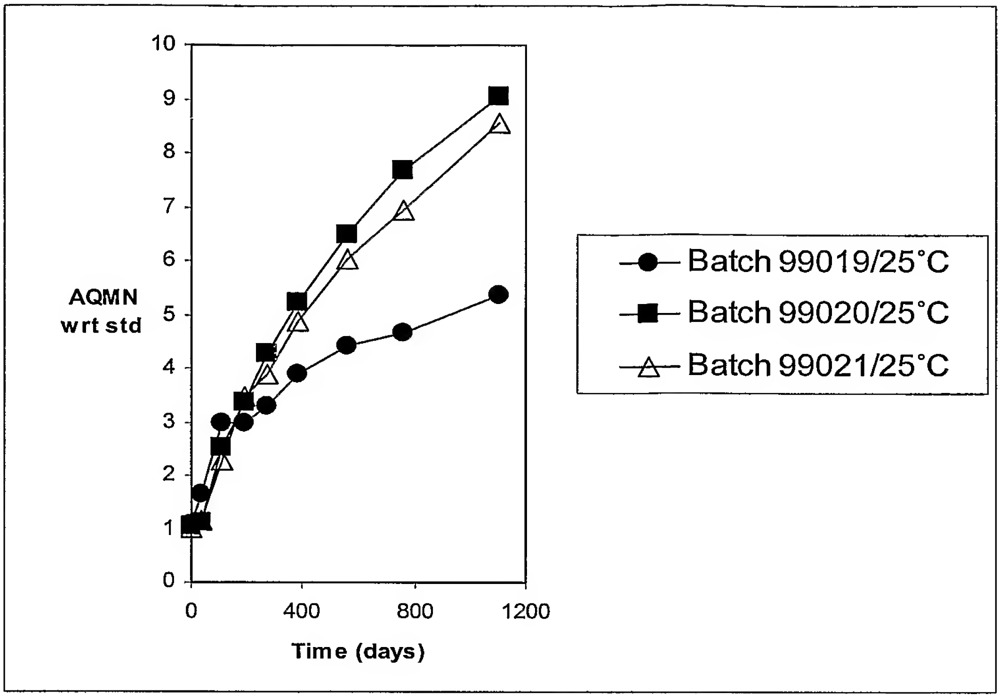

AQMN
wrt std
10
9
8
7
6
5
4
3
2
1
0
0
400
800
1200
Time (days)
Batch 99019/25°C
Batch 99020/25°C
Batch 99021/25°C

Fig. 15

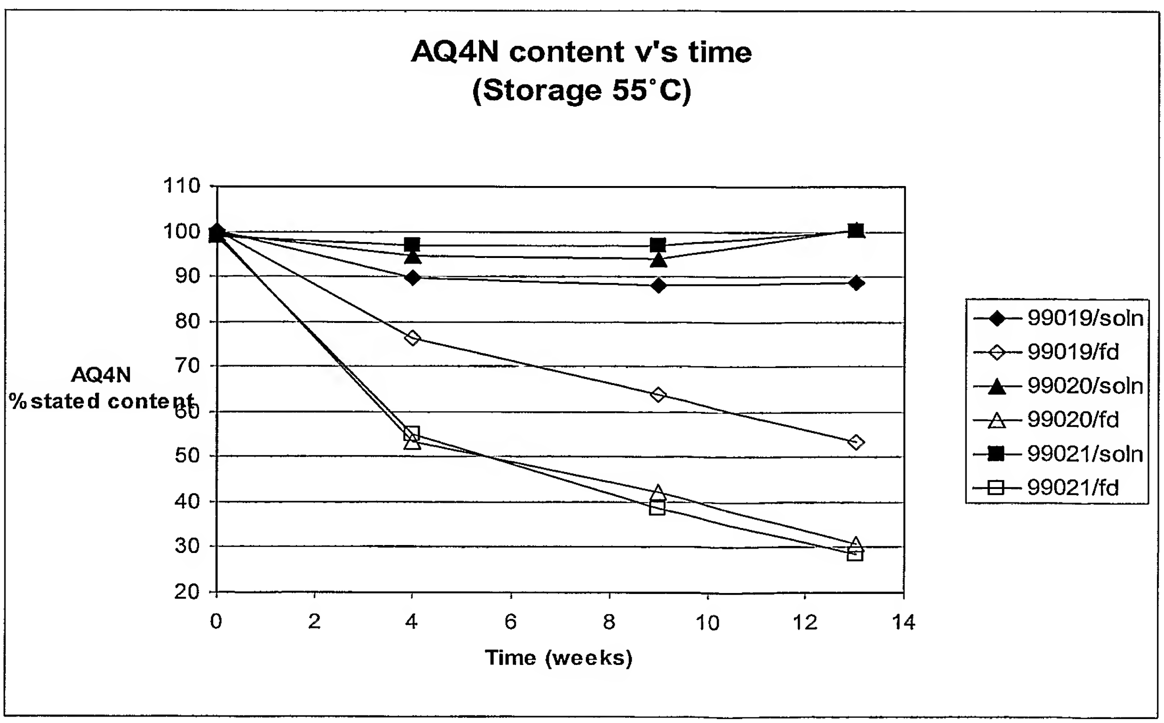

AQ4N content v's time
(Storage 55°C)
AQ4N
% stated content
110
100
90
80
70
60
50
40
30
20
0
2
4
6
8
10
12
14
Time (weeks)
99019/soln
99019/fd
99020/soln
99020/fd
99021/soln
99021/fd

Fig. 16

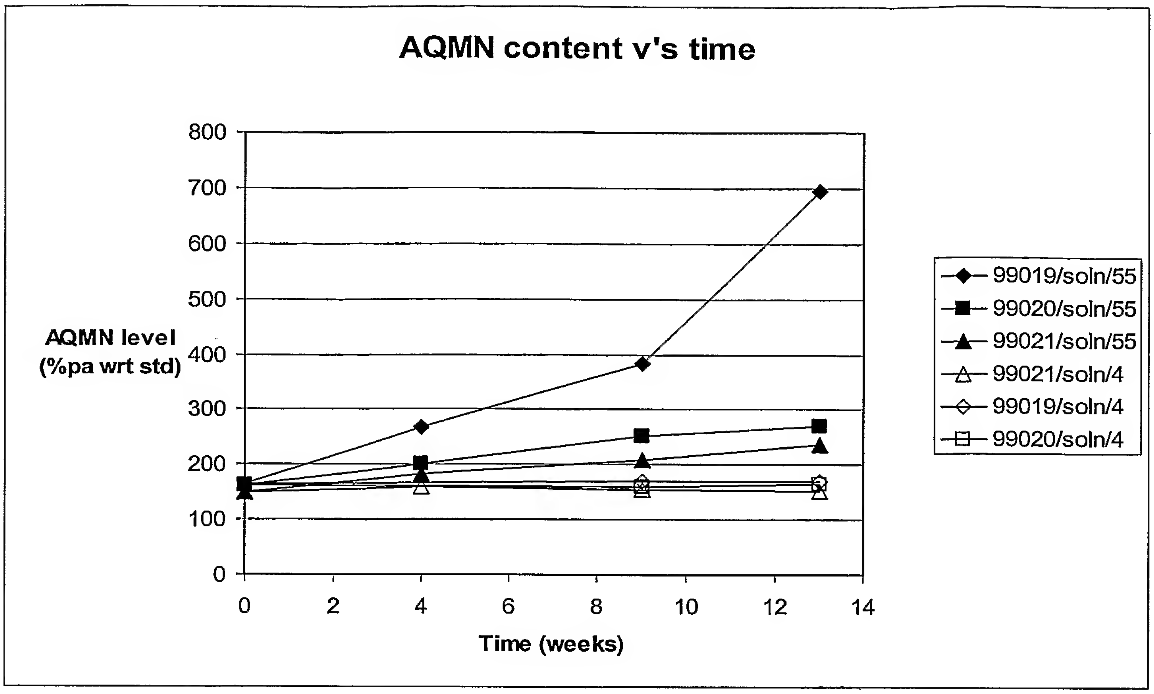

AQMN content v's time
AQMN level (%pa wrt std)
800
700
600
500
400
300
200
100
0
0
2
4
6
8
10
12
14
Time (weeks)
99019/soln/55
99020/soln/55
99021/soln/55
99021/soln/4
99019/soln/4
99020/soln/4

Fig. 17

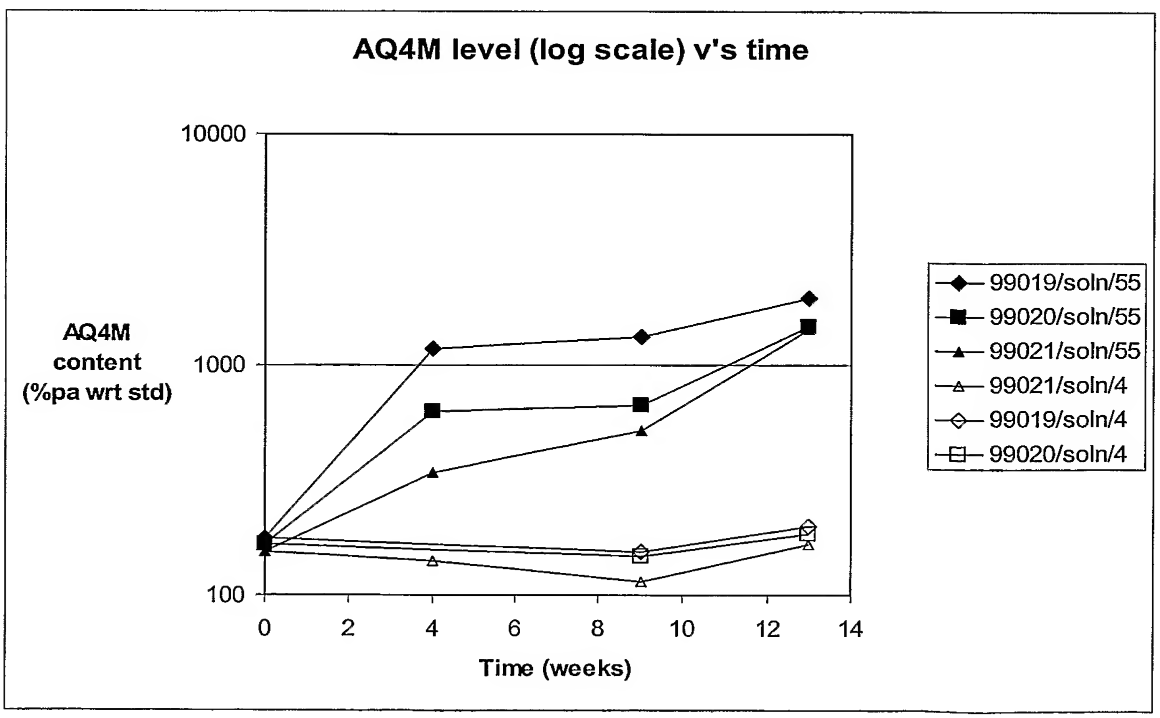

AQ4M level (log scale) v's time
AQ4M content (%pa wrt std)
10000
1000
100
0
2
4
6
8
10
12
14
Time (weeks)
99019/soln/55
99020/soln/55
99021/soln/55
99021/soln/4
99019/soln/4
99020/soln/4

Fig. 18

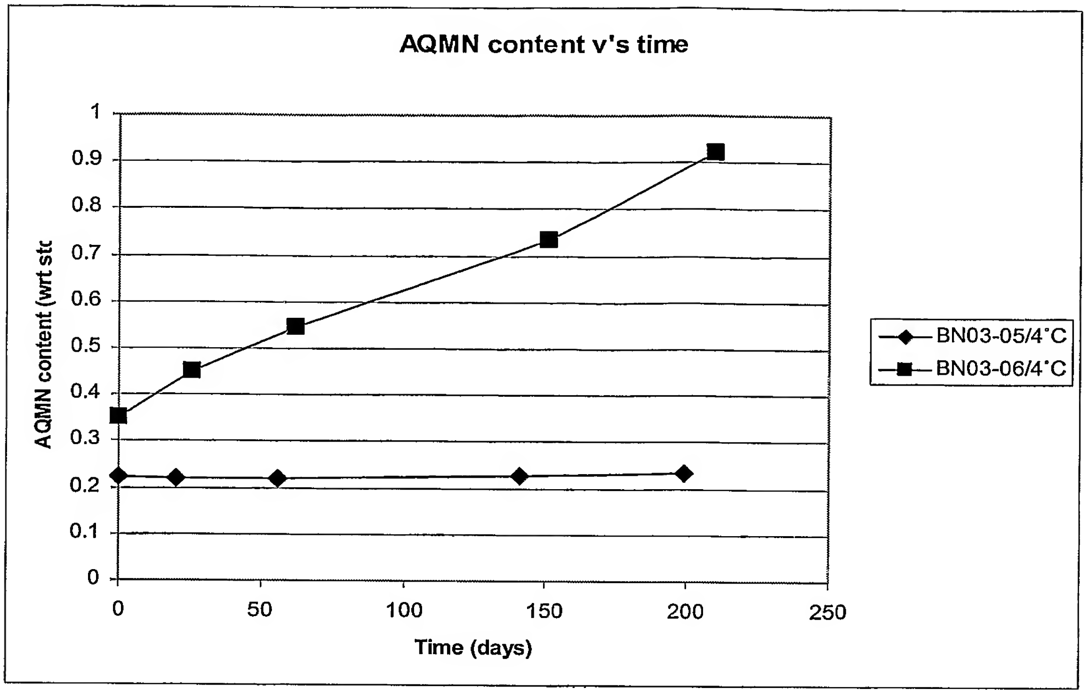

AQMN content v's time
AQMN content (wrt stc
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
50
100
150
200
250
Time (days)
BN03-05/4°C
BN03-06/4°C

Fig. 19

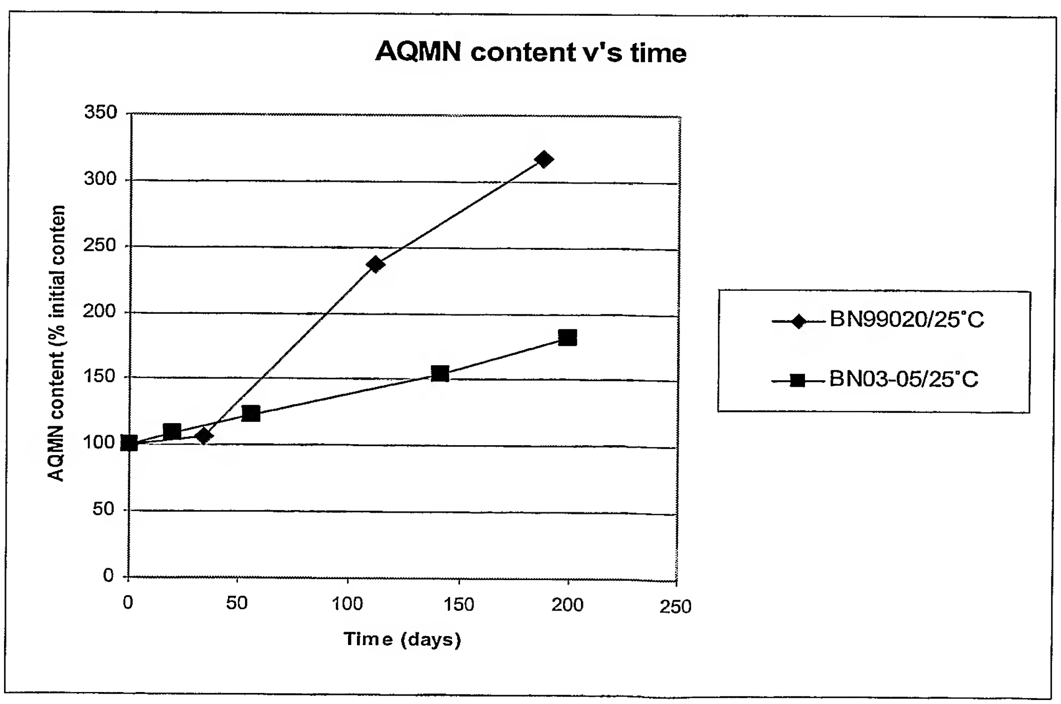

AQMN content v's time
AQMN content (% initial conten
350
300
250
200
150
100
50
0
0
50
100
150
200
250
Time (days)
BN99020/25°C
BN03-05/25°C

Fig. 20

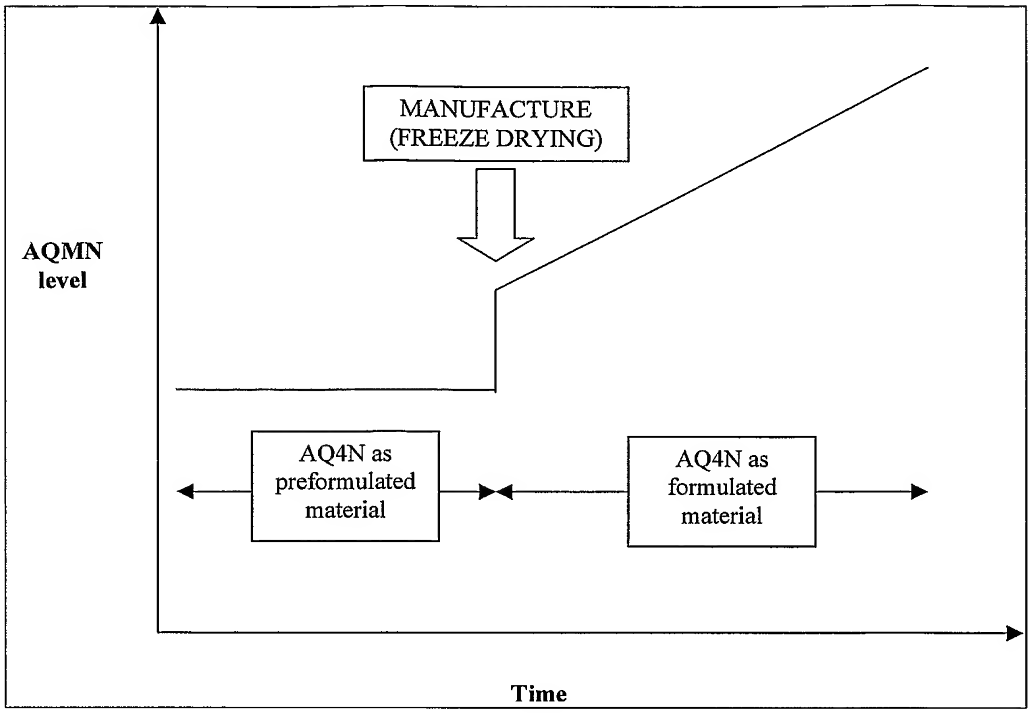

MANUFACTURE
(FREEZE DRYING)
AQMN
level
AQ4N as
preformulated
material
AQ4N as
formulated
material
Time

Fig. 21

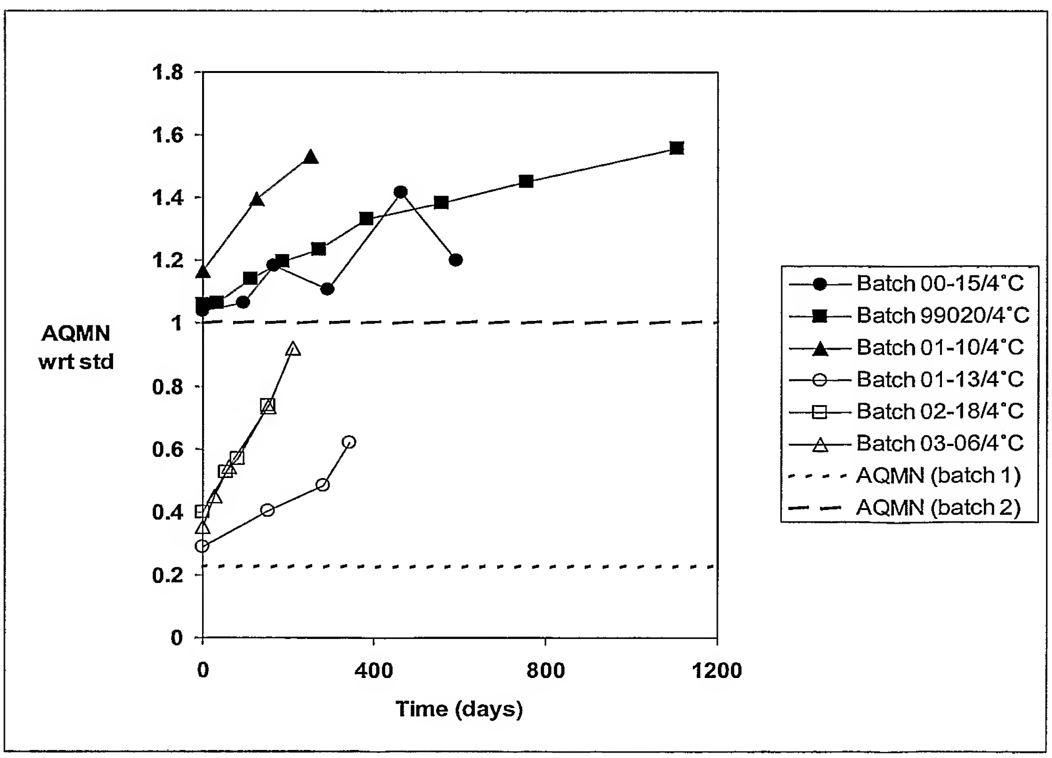

1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
AQMN
wrt std
0
400
800
1200
Time (days)
Batch 00-15/4°C
Batch 99020/4°C
Batch 01-10/4°C
Batch 01-13/4°C
Batch 02-18/4°C
Batch 03-06/4°C
AQMN (batch 1)
AQMN (batch 2)

Fig. 22

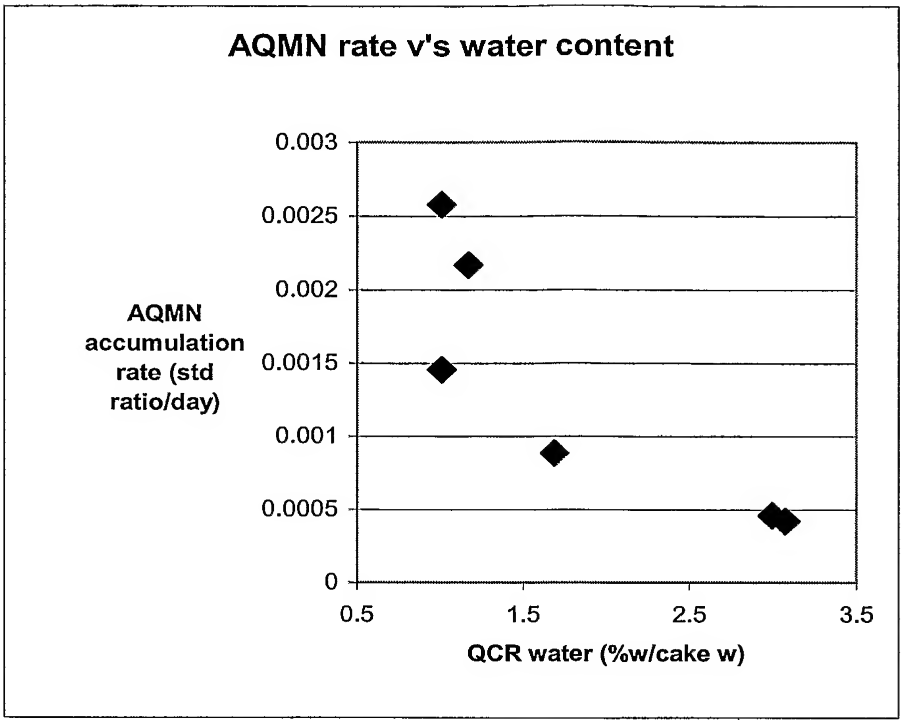

AQMN rate v's water content
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
AQMN accumulation rate (std ratio/day)
0.5
1.5
2.5
3.5
QCR water (%w/cake w)

Fig. 23

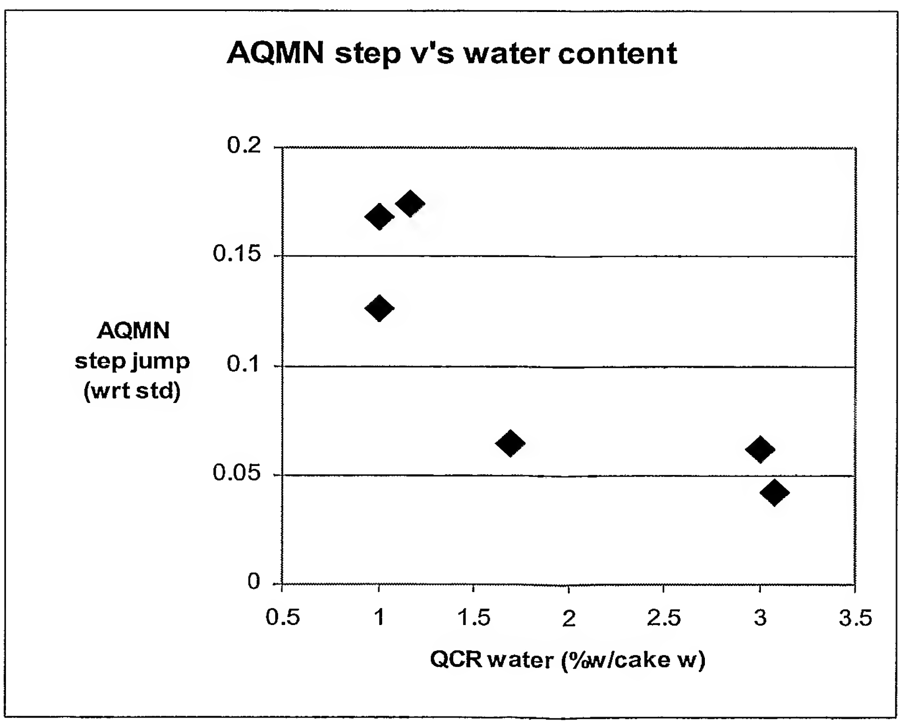

AQMN step v's water content
0.2
0.15
0.1
0.05
0
AQMN step jump (wrt std)
0.5
1
1.5
2
2.5
3
3.5
QCR water (%w/cake w)

Fig. 24

INTERNATIONAL SEARCH REPORT

Application No
/GB2004/003954

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/08 C07C225/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, PAJ, MEDLINE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LOADMAN P M ET AL: "A preclinical pharmacokinetic study of the bioreductive drug AQ4N" DRUG METABOLISM AND DISPOSITION, vol. 29, no. 4 Part 1, April 2001 (2001-04), pages 422-426, XP002306883 ISSN: 0090-9556 page 423, column 1, lines 13-16; table 1	1-12
A	US 5 132 327 A (PATTERSON LAURENCE H) 21 July 1992 (1992-07-21) the whole document	1-12
A	US 6 320 063 B1 (DENNY WILLIAM ALEXANDER ET AL) 20 November 2001 (2001-11-20) the whole document	1-12

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 November 2004	06/12/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Uhl, M

INTERNATIONAL SEARCH REPORT

Application No
/GB2004/003954

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LOADMAN P M ET AL: "Separation methods for anthraquinone related anti-cancer drugs" JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL SCIENCES & APPLICATIONS, ELSEVIER SCIENCE PUBLISHERS, NL, vol. 764, no. 1-2, 25 November 2001 (2001-11-25), pages 193-206, XP004322153 ISSN: 1570-0232 the whole document -----	1-12

INTERNATIONAL SEARCH REPORT

Application No
/GB2004/003954

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